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K+ uptake by fermenting Escherichia coli cells: pH dependent mode of the TrkA system operating.

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Escherichia coli accumulates K+ by means of multiple transport systems, of which TrkA is the most prominent at neutral and alkaline pH while Kup is major at acidic pH. In the present study, K+ uptake was observed with cells grown under fermentative conditions at an initial pH of 9.0 and 7.3 (the medium pH decreased to 8.4 and 6.8, respectively, during the mid-logarithmic growth phase), washed with distilled water and resuspended in a K+ containing medium at pH 7.5 in the presence of glucose. The kinetics for this K+ uptake and the amount of K+ accumulated by the wild type and mutants having a functional TrkA or Kup could confirm that K+ uptake by E. coli grown either at pH 9.0 or pH 7.3 occurs mainly through TrkA. The following results distinguish pH dependent mode of TrkA operating: (1) K+ uptake was inhibited by DCCD in cells grown either at pH 9.0 or pH 7.3, although the stoichiometry of K+ influx to DCCD-inhibited H+ efflux for bacteria grown at pH 9.0 varied with external K+ concentration, but remained constant for cells grown at pH 7.3; (2) K+ uptake was observed with an atpD mutant grown at pH 9.0 but not at pH 7.3; (3) The DCCD-inhibited H+ efflux was increased 8-fold less by 5 mM K+ added into a K+ free medium for bacteria grown at pH 9.0 than that for cells grown at pH 7.3; (4) the DCCD-inhibited ATPase activity of membrane vesicles from bacteria grown at pH 9.0 was reduced a little in the presence of 100 mM K+, but stimulated more than 2.4-fold at pH 7.3.

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Noninvasive tracing of recombinant proteins with "fluorophenylalanine-fingers".

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High-level residue-specific replacement of phenylalanine residues in recombinant human annexin V and azurin from Pseudomonas aeruginosa with o-fluorophenylalanine, m-fluorophenylalanine, and p-fluorophenylalanine has been achieved using the selective pressure incorporation method. Incorporation was confirmed analytically and by UV spectroscopy while the secondary and tertiary structures of these protein mutants in solution remained unchanged upon the effected substitutions. Fluorinated phenylalanines alone and when integrated into proteins exhibit two characteristic and prominent shoulders ("fingers") in the UV spectrum in the range of 260-270 nm, which do not overlap with the contributions of tyrosine and tryptophan residues in the protein UV spectra. Thus, the presence of such "fluorophenylalanine fingers" ("FF fingers") opens a new spectral window to identify the labeled target protein among other nonlabeled cellular proteins in preparative work by simple UV spectroscopy. In the coming era of proteomics such a reliable, cheap, and easy reproducible methodology might have a great potential for speeding up the identification and characterization of target molecules in the total protein output from the genomes of a variety of organisms. Copyright 2000 Academic Press.

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